

Effect of Combined Root Extract of *Sphenocentrum Jollyanum* and *Baphia Nitida* on Some Lipid Profile Indices in Male Wistar Albino rats

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Abstract:-This study is aimed at investigating the effect of combined extract of *Sphenocentrum jollyanum* and *Baphia nitida* on some lipid profile indices in male wistar Albino rats. Seventy (70) Male Wistar rats were divided into seven (7) groups with ten (10) rats per group. Group 1 (control) were fed normal feed and water, Group 2 were treated with 200mg/kg *S.jollyanum*, Group 3 were treated with 200mg/kg *B.nitida*, Group 4 were treated with 200mg/kg *S.Jollyanum*+*B.nitida*, Group 5 were treated with 400mg/kg *S.jollyanum* +*B.nitida*, Group 6 were treated with 400mg/kg *S.jollyanum* and Group 7 were treated with 400mg/kg *B.nitida* via orogastric feeding for 12 weeks. The result was analyzed using One way ANOVA, followed by post hoc multiple comparisons and level of significant set at $p > 0.05$. Randox reagent diagnostic kit was used to perform the test. The results of the investigation revealed that there were no significant differences ($p>0.05$) in the serum cholesterol concentrations between the control rats and the rats treated with different concentrations of *Sphenocentrum jollyanum* and *Baphia nitida* compared to the control. However, the rats treated with 200 and 400mg/kg bw of diherbal mixture of *Sphenocentrum jollyanum* and *Baphia nitida*, although the mean differences were not statistically significant ($p>0.05$). Similarly, there were no significant difference ($p>0.05$) in the serum levels of triglyceride in the extract fed rats when compared with the control. Though elevated levels were recorded in groups fed with 200 and 400mg/kg b.w of diherbal mixture of *S. jollyanum* and *B. Natida* although the mean differences were not significant ($p>0.05$). The HDL result showed that there were significant difference ($p>0.05$) in the serum HDL concentrations between the control rats and the rats treated with 200 and 400mg/kg of diherbal mixture of *Sphenocentrum jollyanum* + *Baphia nitida* compared to the control rats. The change in cholesterol levels may suggest possible effect of high dose and prolonged administration of the extracts which might lead to heart disease.

Keywords: *Sphenocentrum jollyanum*, *Baphia nitida*, cholesterol, triglyceride and orogastric

I. INTRODUCTION

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world.

Medicinal plants have been identified and used throughout human history. Plants make many chemical compounds that are for biological functions, including defence against insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total [6]. Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to have beneficial pharmacology, but also gives them the same potential as conventional pharmaceutical drugs to cause harmful side effects [10]. Moreover, plant material comes with a variety of compounds which may have undesired effects, though these can be reduced by processing. The use of plants as medicines pre-dates written human history. *Sphenocentrum jollyanum*, and *Baphia nitida* happens to be one of such medicinal plants utilized for sexual performances.

Sphenocentrum jollyanum (*Menispermaceae*) is a perennial plant that grows naturally along the west coast sub region of Africa with expanse from Cameroon across Nigeria to Sierra Leone. *Sphenocentrum jollyanum* is an evergreen shrub growing around 1.5 metres tall and has been in traditional medicine practice for centuries with its leaves, roots and latex all in use [2].

Baphia nitida (camwood, also barwood), also known as African sandalwood, is a shrubby, leguminous, hard-wooded tree from central West Africa. This wood is of a very fine colour, and is used in woodturning for making knife handles and similar articles. The tree's bark and heartwood are commonly used to make a brilliant but non-permanent red dye, which is soluble in alkali [3].

II. MATERIALS AND METHODS

Materials: Syringes, Filter paper, Electronic weighing balance (Arman, England), orogastric cannula, plain and EDTA sample bottles, normal saline, chloroform anesthesia, water bath (Kutterman, U.K), bucket centrifuge machine (Labman, U.K), Spectrophotometer (Jenway, England), Dissecting kits (Haweley, England) and rat specific kits for liver enzymes.

Ethanoic Preparation of Sphenocentrum jollyanum and Baphia nitida root

The fresh root of *Sphenocentrum jollyanum* and *Baphia nitida* were collected and sent to a Botanist in Pharmacology Department Madonna University Nigeria for identification. They were chopped into pieces and air dried (36-39° C). The dried root was ground to a coarse powder with grinder and stored in a closed earthenware pot which was left open when placed on a fire without adding water. The 1000g and 500g of the powered *Sphenocentrum jollyanum* and *Baphia nitida* respectively were extracted with 100% ethanol within 72h. The ethanol filtrate was concentrated to obtain a crude extract and evaporated to dryness in water bath at 60°C for 36hours. The brownish residue were weighed and kept in an air tight bottle in the refrigerator until use. This method was recently used by [7],

Laboratory animals

Seventy (70) male albino Wistar rats aged 8 weeks and weighing 150–200g were used for this study. The animals were housed in the Department of Biochemistry Animal house, Madonna University, Elele, Rivers State, Nigeria. Standard animal cages with wood dust as bedding were used in keeping the animals. They were allowed ad libitum access to mice specific feed and clean water, and exposed to 12/12-hr light/dark cycle. The animals were acclimatized for 7 days..

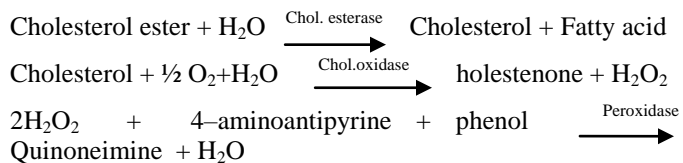
Experimental Design

Group 1(control) were fed normal feed and water, Group 2 were treated with 200mg/kg *S.jollyanum* ,Group 3 were treated with 200mg/kg *B.nitida*, Group 4 were treated with 200mg/kg *S.Jollyanum+B.nitida*, Group 5 were treated with 400mg/kg *S.jollyanum +B.nitida*, Group 6 were treated with 400mg/kg *S.jollyanum* and Group 7 were treated with 400mg/kg *B.nitida* crude extract and this was done via orogastric feeding once daily for 60 days after which the animals were sacrificed under chloroform anaesthesia and blood sample was collected for liver enzyme assay. Method of administration recently used by, Wopara, *et al*, 2019. [12].

Determination of total cholesterol concentration

The concentration of cholesterol was determined according to the method of Allain *et al.* (1976).[1].

Principle: Cholesterol esterase hydrolyses esterified cholesterol to free cholesterol. The free cholesterol is oxidized to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrene by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex as shown in the reactions below;



Reagents: Biosystem cholesterol kit.

Reagents A: (35 mMol/l) Sodium cholate, phenol 28 mmol/l, Cholesterol esterase > 0.2 U/ml, Cholesterol oxidase > 0.4 U/l, peroxidase > 0.8 U/ml; 0.5 mmol/l, 4-aminoantipyrene, pH 7.0

Procedure: Three test tubes were prepared for the reactions as follows;

Reagent	Blank	Standard	Sample
Cholesterol	--	10 µl	--
Sample	--	-	10 µl
Reagent A	1.0 ml	1.0 ml	1.0 ml

The test tubes were mixed thoroughly and incubated for 10 minutes at room temperature.

The Absorbance of samples (A_{samples}) and standard (A_{standard}) were read against the reagent blank at 500 nm using a Turner^(R) 390 spectrophotometer.

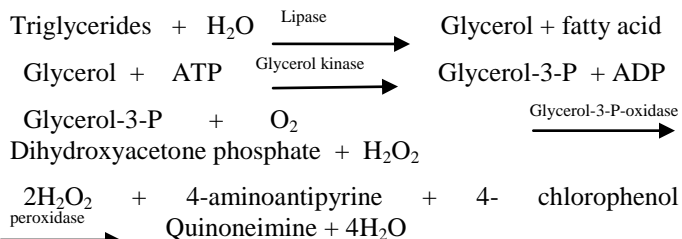
Calculations: Cholesterol concentrations in the sample were calculated as follows:

$$C_{\text{Sample}} = \frac{A_{\text{sample}} - A_{\text{sample blank}} \times C_{\text{standard}}}{A_{\text{standard}}}$$

Determination of triacylglycerides concentration (TG)

The concentration of triacylglycerides was determined according to the method of Young (1995)[14]

Principle: Lipoprotein lipase hydrolyses triacylglycerides to glycerol and free fatty acids. The glycerol formed reacts with ATP in the presence of glycerol kinase forming glycerol-3-phosphate which is oxidized to form hydrogen peroxide. The hydrogen peroxide further reacts with phenolic compound (chlorophenol) and 4- aminoantipyrene by the catalytic action of a peroxidase to form a red coloured quinoneimine dye complex.



Reagents: TG standard (glycerol 200 mg/dl)

Reagent A (45 mmol/L glycerol kinase > 4 Umol/l; peroxidase> 0.8 Umol/l, 4-aminoantipyrene, 0.75 mmol/L, ATP µmol/ml, pH 7.0

Procedure:

Reagents	Blank	Standard	Sample
TG standard	--	10 µl	--
Sample	--	--	10 µl

Reagent A 1.0 ml 1.0 ml 1.0 ml
 The test tubes were mixed thoroughly and incubated for 15 minutes at room temperature.

The absorbance of samples (A_{samples}) and standard (A_{standard}) were read against the reagent blank at 500 nm in a Turner^(R) 390 spectrophotometer.

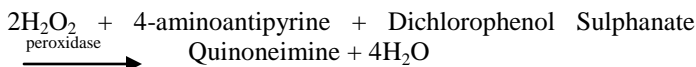
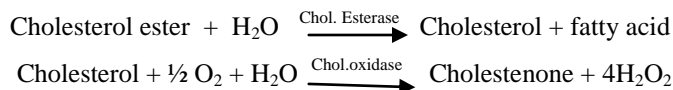
Calculations: The cholesterol concentration of the sample was calculated as follows:

$$C_{\text{Sample}} = \frac{A_{\text{sample}} - A_{\text{sample blank}} \times C_{\text{standard}}}{A_{\text{standard}}}$$

Determination of high density lipoprotein (HDL)-cholesterol concentration

The concentration of HDL-cholesterol was determined according to the method of Grove, 1979.[5]

Principle: Very low-density Lipoprotein (VLDL) and low-density lipoprotein (LDL) in the sample precipitate with phosphotungstate and magnesium ions. On centrifugation, all HDL present in the supernatant is measured spectrophotometrically by means of the coupled reactions described below;



Reagents; HDL cholesterol standard (S), Reagent B; 35 mmol/l phosphate buffer, 0.5 mmol/l sodium cholate, cholesterol esterase > 0.2 U/ml, cholesterol oxidase > 0.1U/L, peroxidise > 1.0 U/ml; 0.5 mmol/l 4-aminoantipyrine, 4.0 mmol/l dichlorophenol sulphanate, pH 7.0

Reagent A – Phosphotungstate 0.4 mmol/l and 20 mmol/l Magnesium chloride.

Procedure: Reagents were pipetted into labelled test tubes as follows;

Sample (serum) 0.2 ml
 Reagent A 0.5 ml

The test tubes were mixed thoroughly and stood at room temperature for 10 minutes. After centrifugation for 10 minutes at 4000 rpm, supernatants were carefully collected into labelled test tubes as follows;

Reagents	Blank	Standard	Sample
Distilled water	50 ul	--	--
HDL cholesterol standard (S)	--	50 ul	--
Sample supernatant	--	--	50 ul
Reagent B	1.0 ml	1.0ml	1.0 ml

The test tubes were mixed thoroughly and incubated at room temperature for 30 minutes. The absorbance of samples (A_{samples}) and standard (A_{standard}) were read against the reagent blank at 500 nm in a Turner 390 spectrophotometer.

Calculations: Cholesterol concentration in the sample (C_{sample}) was calculated as follows.

$$C_{\text{Sample}} = \frac{A_{\text{sample}} - A_{\text{sample blank}} \times C_{\text{standard}}}{A_{\text{standard}}}$$

III. RESULTS

THE EFFECT OF 200 AND 400MG/KG BODY WEIGHT (B.W.) OF *Sphenocentrum jollyanum* AND *Baphia natida* ON CHOLESTEROL CONCENTRATION IN Mmol/L OF MALE RATS AFTER 60 DAYS

Figure 1 below reveals that there were no significant differences ($p>0.05$) in the serum cholesterol concentrations between the control rats and the rats treated with different concentrations of *Sphenocentrum jollyanum* and *Baphia natida* compared to the control. However, the rats treated with 200 and 400mg/kg bw of diherbal mixture of *Sphenocentrum jollyanum* and *Baphia natida* (73.81 ± 5.10 and $78.92 \pm 9.57\text{mmol/L}$) respectively, although the mean differences were not statistically significant ($p>0.05$).

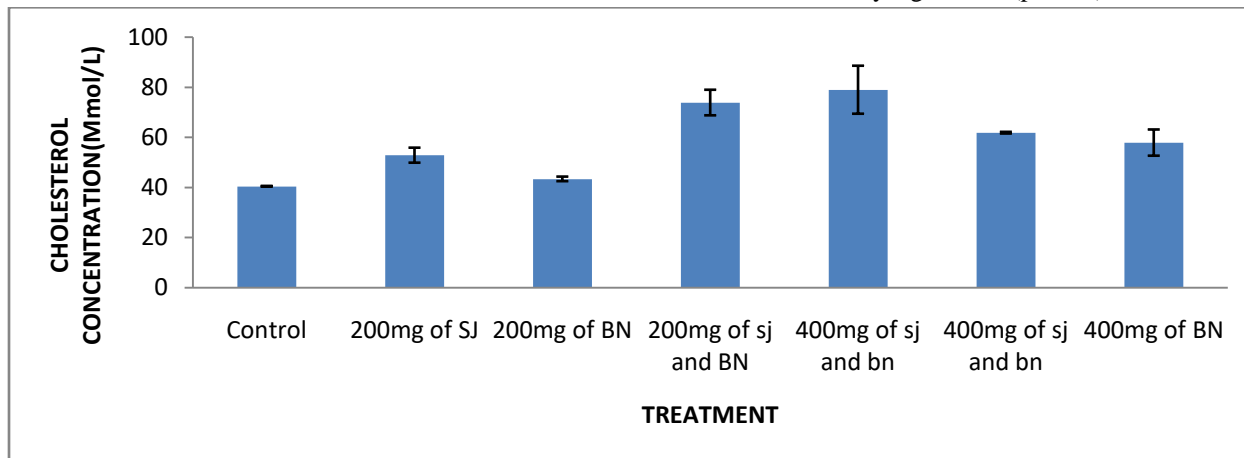


Fig. 1: Serum cholesterol absorption of male wistar rats fed with *S. jollyanum* and *B. nitida*

Each plot represents the mean ± SEM (n=10)

THE EFFECT OF 200 AND 400MG/KG BODY WEIGHT (B.W.) OF *Sphenocentrum jollyanum* AND *Baphia natida* ON TRIGLYCERIDE IN MMOL/L OF MALE RATS AFTER 60 DAYS.

Figure 2 underneath revealed that there were no significant difference ($p>0.05$) in the serum levels of triglyceride in the

extract fed rats when compared with the control. Though elevated levels were recorded in groups fed with 200 and 400mg/kg b.w of diherbal mixture of *S. jollyanum* and *B. natida* (79.54 ± 15.95 and 105.76 ± 0.45 mmol/L), respectively (5.32 ± 0.39 mmol/L), although the mean differences were not significant ($p>0.05$).

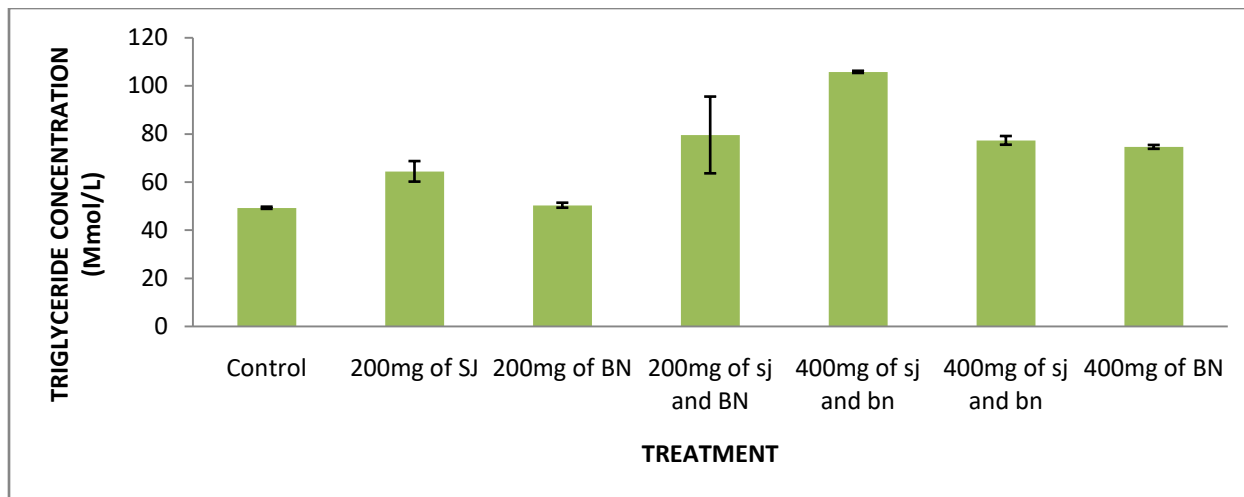


Fig 2: Serum Triglyceride absorption of male wistar rats fed with *S. jollyanum* and *B. nitida*

Each plot represents the mean \pm SEM (n=10)

THE EFFECT OF 200 AND 400MG/KG BODY WEIGHT (B.W.) OF *Sphenocentrum jollyanum* AND *Baphia natida* ON HIGH DENSITY LIPOPROTEIN (HDL) IN MMOL/L OF MALE RATS AFTER 60 DAYS

Figure 3 below reveals that there were significant difference ($p>0.05$) in the serum HDL concentrations between the

control rats and the rats treated with 200 and 400mg/kg of diherbal mixture of *Sphenocentrum jollyanum* + *Baphia natida* (93.20 ± 0.37 and 91.40 ± 0.20 mmol/L) respectively compared to the control rats (272.2 ± 0.20 mmol/L),

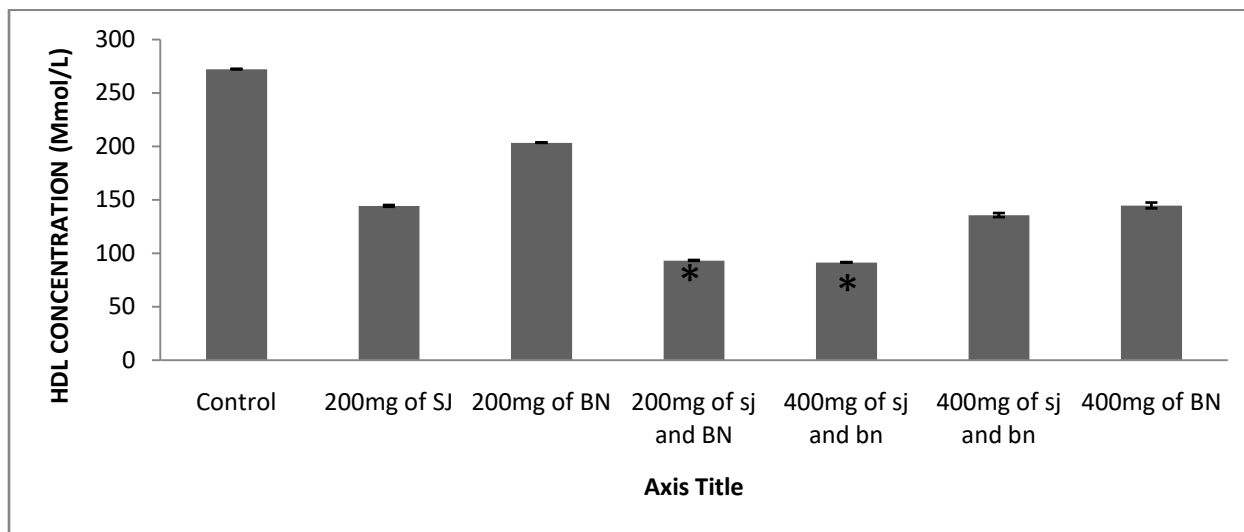


Fig 3: Serum High density lipoprotein cholesterol absorption of male wistar rats administered *Sphenocentrum jollyanum* and *Baphia natida*

Each plot represents the mean \pm SEM (n=10)

* Significantly different ($p<0.05$) from the control

IV. DISCUSSION AND CONCLUSION

Changes in the serum concentration of key lipids like high density lipoprotein, triglyceride and cholesterol may possibly be due to elevated mobilization of lipids which may likely lead to hyperlipidemia and coronary arterial disease [13]. High density lipoprotein cholesterol protects beside CHD. The risk of CHD from arteriosclerosis is inversely proportional to serum level of HDL [4]. Higher triglyceride is connected with stroke, diabetes, obesity which may likely be implicated in hyperthyroidism, cirrhosis, low protein, hyperlipoproteinemia and elevated carbohydrate diet, badly regulated diabetes mellitus and pancreatitis [8].

Low level of triglyceride specifies malnutrition, malabsorption, hyperthyroidism, low fats diet and terminal ailment [8].

It was observed from the result that there was an elevated level of lipids in groups fed with 200mg/kg and 400mg/kg of *Sphenocentrum jollyanum*, 200mg/kg and 400mg/kg of the combined extract and the 400mg/kg body weight of *Baphia nitida* compared to control group. Although, there was non significant ($P < 0.05$) increase. Cholesterol is also a vital requirement for normal testicular activity [11], hence increase in the steroid hormone (testosterone) of male rats used in the experiment.

Triglyceride level after the 60 days administration for both extract showed that 400mg/kg body weight of diherbal mixture significantly ($p < 0.05$) increased the level of triglyceride when compared with the control. Similarly, there was increase in triglyceride level in all other groups, although the increase was not statistically significant ($p < 0.05$) when compared with the control. Elevated triglyceride is connected with high risk for atherosclerosis and may lead to heart attack [8].

It was recorded that after the ingestion of the extracts, the concentration of HDL cholesterol decrease in all the experimental groups compared to the control. Though, the mean levels of the group fed with combined extract had significant ($p < 0.05$) reduction compared with other experimental groups and control. The reduction in HDL cholesterol may probably be an indication of cardiovascular risk [9].

Conclusion

This study showed the regular intake of these extracts *S. jollyanum* and *B. nitida* can increase the level of cholesterol which may be beneficial in the synthesis of steroid hormones and in excess may lead to heart disease also long administration of the extracts reduced the good cholesterol (HDL) which is also a cause for concern.

REFERENCES

- [1]. Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W. & Fu, P.C. (1976). Enzymatic determination of total cholesterol. *Clin Chem.* 20(4), 470-475.
- [2]. Burkill, H.M. (1997). The useful plants of West Tropical Africa. 2nd Edition. Volume 4, Families M–R. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 969 pp
- [3]. Gill, L.S. (1992). Ethnomedical uses of plants in Nigeria, Benin-City, Uniben Press. 134-138.
- [4]. Gordon, D.J. & Rifkind, B.M. (1989). High density lipoprotein. The clinical implications of recent studies. *New England Journal of Medicine*, 321:1311-1316.
- [5]. Grove, T.H (1979). Effect of reagent pH on determination of high-density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. *Clin Chem.* 25, 560-564.
- [6]. Lichterman, B. L. (2004). "Aspirin: The Story of a Wonder Drug". *British Medical Journal*, 329 (7479): 1408
- [7]. Mbaka, G.O and Owolabi, M.A (2011). Evaluation of haematinic activity and subchronic toxicity of *sphenocentrum jollyanum* menispermaceae seed oil, *Eur.J.med.plants.* 1(4): 140-152
- [8]. Ochei, J. & Kolhatkar, A. (2008). Lipid and lipoprotein. In *Medical laboratory science, theory and practice.* Tata McGraw – Hill, New Delhi.
- [9]. Panagiotakos, B., Pitsavos, C., Skoumas, J., Chrysoschosu, C., Toutouza, M., Stefanadis, C.I. & Toutouzas, P.K. (2003). Importance of LDL/HDL ratio heterozygous familial hypercholesterolemia: A 15 years following (1987 – 2002). *Current Medical Research and Opinion*, 19:89.
- [10]. Tapsell, L.C., Hemphill, I., Cobiac, L. (2006). "Health benefits of herbs and spices: the past, the present, the future". *The Medical Journal of Australia.* 185 (4): 4–24.
- [11]. Watcho, P., Kamatchouing, P., Sokeng, S.D., Moundipa, P.F., Tantchou, J., Essame, J.L. & Koueta, N. (2004). Androgenic effect of *Mondia whitei* roots in male rats. *Asian Journal of Andrology*, 6:269-272.
- [12]. Wopara, Iheanyichukwu, Uwakwe, A. A, Wegwu, M. O, Mobisson, Samuel Kelechi & Emefu, Chinwe Queen (2019). The Evaluation of Combined Crude Root Extract of *Sphenocentrum jollyanum* and *Baphia nitida* on Some Liver Enzymes in Male Wistar Albino Rats. *World Wide Journal of Multidisciplinary Research and Development*, 5(3): 11-14
- [13]. Yakubu, M.T., Akanji, M.A. & Oladiji, A.T. (2008). Alterations in serum lipid profile of male rats by oral administration of aqueous extract of *Fadogia agrestis* stem. *Research Journal of Medical Plant*, 2:66.
- [14]. Young D.S. (1995). *Effect of Drug on Clinical Laboratory Tests*, 4th Ed. AACC Press.